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Note

Ethyl 4-[(1-substituted indol-2-yl)methoxy]benzoates and indoline derivatives: Anti-juvenile hormone and juvenile hormone activities

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A number of ethyl 4-[(1-substituted indol-2-yl)methoxy]benzoates and indoline derivatives were prepared as rigid congeners of ethyl 4-(2-benzylhexyloxy)benzoate (KF-13), an anti-juvenile hormone (anti-JH) agent, and tested for both anti-JH and JH activities in silkworm larvae. In contrast to KF-13, the precocious metamorphosis-inducing activity of which decreased by increasing the applied doses, 1-*n*-propyl, 1-*n*-butyl (**1c**) and 1-benzyl (**1d**) derivatives were found to induce higher percentages of precocious metamorphosis at high doses. Compounds **1c** and **1d** also exhibited JH activity when topically applied to allatectomized 4th instar larvae. Ethyl 4-[(*S*)-(1-*n*-butylindolin-2-yl)methoxy]benzoate, which showed precocious metamorphosis-inducing activity at high doses, had no JH activity.

Keywords: anti-juvenile hormone, juvenile hormone, indole, indoline, precocious metamorphosis.

Introduction

Since juvenile hormone (JH) is involved in a wide range of physiological processes in insects such as metamorphosis, reproduction and diapause,[1] anti-JH agents, which chemically block the functioning of the JH control system, would be potentially useful not only as biochemical probes to assist in elucidating the role of JH in insect development and reproduction, but also as insect growth regulators.[2] We have recently found a novel anti-JH agent, ethyl 4-(2-benzylhexyloxy)benzoate (KF-13),[3] by modifying the structure of ethyl 4-[2-(*tert*-butylcarbonyloxy)-butyloxy]benzoate (ETB), which is the only compound reported to act as a partial JH antagonist in the larval epidermis of *Manduca sexta in vitro*.[4] ETB is also known to have both JH and anti-JH activities, depending on the doses applied.[5] KF-13 showed much stronger precocious metamorphosis-inducing (anti-JH) activity than ETB, but the JH activity of KF-13 was less than that of ETB.[6] KF-13 induced precocious metamorphosis at relatively low doses; however, at higher doses, its activity markedly decreased, probably due to the counteraction caused by KF-13 itself as a JH agonist. The anti-JH activity of KF-13 was completely counteracted by methoprene, a JH agonist, not by 20-hydroxyecdysone.[3] In our continuing studies of this series of compounds, we designed and synthesized indole derivatives **1**, in which the chiral carbon portion of KF-13 is rigidified (Fig. 1). In the present paper, we report anti-JH and JH activities of a novel series of ethyl 4-[(1-substituted indol-2-yl)methoxy]benzoates and related indoline derivatives.

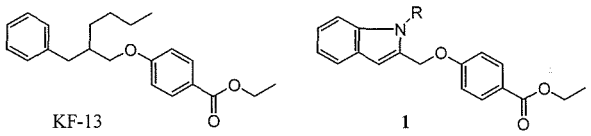

Fig. 1. Structures of KF-13 and indole derivatives **1**.

Materials and Methods

1. Instrumental analysis and chemicals

^{1}H NMR spectra were determined with a JEOL EX-400 (400 MHz) spectrometer, using tetramethylsilane as an internal standard, and all samples were prepared in deuterochloroform. Methoprene (93.4%) was kindly supplied by Earth Biochemical Co. The preparation of a series of ethyl 4-[(1-substituted indol-2-yl)methoxy]benzoates and related (*S*)-indoline derivatives is outlined in Fig. 2(A) and (B), respectively.

*1.1. Methyl 1-ethylindole-2-carboxylate (**II**: R=C_2H_5)*

A solution of indole-2-carboxylic acid (1.0 g, 6.2 mmol) in 15 ml methanol containing a few drops of H_2SO_4 was refluxed for 12 hr. After removing the solvent under reduced pressure, the residue was dissolved in ethyl acetate and the ethyl acetate solution was washed with 2M aq. NaOH solution and brine, and dried over Na_2SO_4. The concentration of the organic layer gave 1.06 g (97%) crude methyl indole-2-carboxylate.

To a solution of the above methyl indole-2-carboxylate (0.30 g, 1.7 mmol) in 5 ml DMSO was added potassium *tert*-butoxide (0.29 g, 2.6 mmol) and ethyl bromide (0.28 g, 2.6 mmol). After stirring for 16 hr at room temperature, the product was extracted with 100 ml ethyl acetate. The ethyl acetate solution was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel by eluting with hexane–ethyl acetate (9 : 1) to give 0.31 g (99%) of **II** (R=C_2H_5) as a colorless oil. ^{1}H NMR δ: 1.45 (3H, t, J=7.3 Hz, CH_3), 3.66 (3H, s, OCH_3), 4.48 (2H, q, J=7.3 Hz, CH_2), 7.16 (1H, t, J=7.3 Hz, indolyl), 7.26 (1H, S, indoyl), 7.29 (1H, t, J=7.3 Hz, in-

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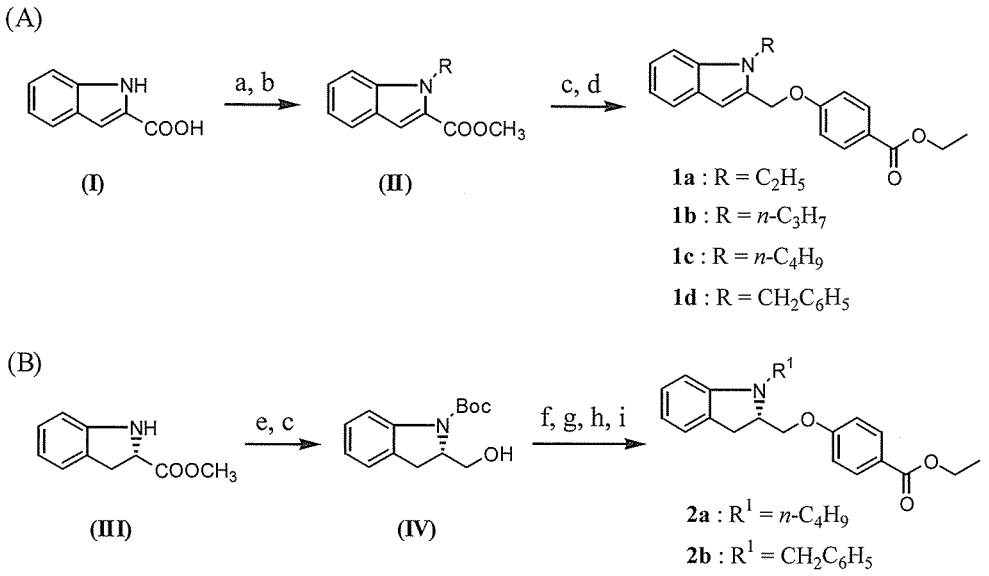

Fig. 2. Synthetic scheme for the preparation of (A) indole derivatives and (B) indoline derivatives. (a) H_2SO_4, MeOH; (b) KO-*tert*-C_4H_9, alkyl bromide or iodide, DMSO; (c) $LiAlH_4$, THF; (d) $(C_6H_5)_3$ P, ethyl 4-hydroxybenzoate, diisopropyl azodicarbonate, DMF; (e) di-*tert*-butyldicarbonate, THF; (f) *p*-toluenesulfonyl chloride, triethylamine, 4-dimethylaminopyridine, CH_2Cl_2; (g) NaH, ethyl 4-hydroxybenzoate, DMF; (h) CF_3COOH, CH_2Cl_2; (i) *n*-C_4H_9Br or benzyl bromide, K_2CO_3, DMF.

dolyl), 7.34 (1H, d, J=7.3 Hz, indolyl), 7.59 (1H, d, J=7.3 Hz, indolyl).

*1.2. Ethyl 4-[(1-ethylindol-2-yl)methoxy]benzoate (**1a**)*

To a suspension of lithium aluminum hydride (0.04 g, 1.3 mmol) in 10 ml THF at 0°C was added **II** (R=C_2H_5, 0.20 g, 1.0 mmol) and the mixture was stirred for 1 hr at room temperature. The reaction mixture was quenched with saturated NH_4Cl solution and filtrated. After removing the solvent under reduced pressure, the residue was extracted with ethyl acetate. The ethyl acetate solution was washed with brine and dried over Na_2SO_4. The concentration of the organic layer gave 0.16 g (80%) crude 1-ethylindole-2-methanol.

To a solution of the above alcohol (0.20 g, 1.1 mmol) in 10 ml DMF was added triphenylphosphine (0.33 g, 1.3 mmol), ethyl 4-hydroxybenzoate (0.21 g, 1.3 mmol), and 40% diisopropyl azodicarbonate in toluene (0.63 g, 1.3 mmol). After stirring for 16 hr at room temperature, the product was extracted with ethyl acetate. The ethyl acetate solution was washed with 2 M aq. NaOH solution and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel by eluting with hexane–ethyl acetate (6 : 1) to give 0.10 g (54%) of **1a** as a colorless oil. ^{1}H NMR δ: 1.39 (3H, t, J=7.3 Hz, CH_3), 1.42 (3H, t, J=7.3 Hz, CH_3), 4.24 (2H, q, J=7.3 Hz, CH_2), 4.36 (2H, q, J=7.3 Hz, CH_2), 5.32 (2H, s, CH_2), 6.61 (1H, S, indoyl), 7.04 (2H, d, J=8.8 Hz, phenyl), 7.12 (1H, t, J=7.3 Hz, indolyl), 7.25 (1H, t, J=7.3 Hz, indolyl), 7.33 (1H, t, J=7.3 Hz, indolyl), 7.64 (1H, d, J=7.3 Hz, indolyl), 8.03 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 74.28; H, 6.55; N, 4.51%. Calcd. for $C_{20}H_{21}NO_3$: C, 73.62; H, 6.59; N, 4.33%.

Compounds **1b–1d** were prepared in the same manner as **1a** using the corresponding alkyl bromide or iodide instead of ethyl bromide.

*Ethyl 4-[(1-n-propylindol-2-yl)methoxy]benzoate (**1b**)*

^{1}H NMR δ: 0.96 (3H, t, J=7.3Hz, CH_3), 1.35–1.44 (2H, m, CH_2), 1.38 (3H, t, J=7.3Hz, CH_3), 1.87 (2H, m, CH_2), 4.11–4.16 (2H, m, CH_2), 4.35 (2H, q, J=7.3 Hz, CH_2), 5.28 (2H, s, CH_2), 6.61 (1H, S, indoyl), 7.04 (2H, d, J=8.8 Hz, phenyl), 7.11 (1H, t, J=7.3 Hz, indolyl), 7.25 (1H, t, J=7.3 Hz, indolyl), 7.34 (1H, d, J=7.3 Hz, indolyl), 7.62 (1H, d, J=7.3 Hz, indolyl), 8.03 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 74.57; H, 6.93; N, 4.73%. Calcd. for $C_{21}H_{23}NO_3$: C, 74.75; H, 6.87; N, 4.15%.

*Ethyl 4-[(1-n-butylindol-2-yl)methoxy]benzoate (**1c**)*

^{1}H NMR δ: 0.93 (3H, t, J=7.3 Hz, CH_3), 1.35–1.44 (2H, m, CH_2), 1.39 (3H, t, J=7.3Hz, CH_3), 1.83 (2H, quin, J=7.3Hz, CH_2), 4.24 (2H, t, J=7.3 Hz, CH_2), 4.36 (2H, q, J=7.3 Hz, OCH_2), 5.25 (2H, s, CH_2), 6.61 (1H, S, indoyl), 7.04 (2H, d, J=8.8 Hz, phenyl), 7.12 (1H, t, J=7.3 Hz, indolyl), 7.24 (1H, t, J=7.3 Hz, indolyl), 7.35 (1H, d, J=7.3 Hz, indolyl), 7.61 (1H, d, J=7.3 Hz, indolyl), 8.03 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 75.17; H, 7.37; N, 4.62%. Calcd. for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99%.

*Ethyl 4-[(1-benzylindol-2-yl)methoxy]benzoate (**1d**)*

^{1}H NMR δ: 1.37 (3H, t, J=7.3 Hz, CH_3), 4.36 (2H, q, J=7.3 Hz, CH_2), 5.25 (2H, s, CH_2), 5.46 (2H, s, CH_2), 6.69 (1H, S, indoyl), 6.86 (2H, d, J=8.8 Hz, phenyl), 6.92–6.98 (2H, m, indolyl), 7.15 (1H, t, J=7.3 Hz, indolyl), 7.22–7.29 (6H, m, indolyl and phenyl), 7.65 (1H, d, J=7.3 Hz, indolyl), 8.00 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 77.48; H, 6.01; N, 3.70%. Calcd. for $C_{25}H_{23}NO_3$: C, 77.90; H, 6.01; N, 3.63%.

*1.3. (S)-1-(t-butyloxycarbonyl)indoline-2-methanol (**IV**)*

Methyl (*S*)-indoline-2-carboxylate (**III**) was prepared from (*S*)-indoline-2-carboxylic acid in the same manner as methyl indole-2-carboxylate.

A solution of (**III**) (0.94 g, 5.3 mmol) and di-*tert*-butyl dicar-

bonate (13.4 g, 6.0 mmol) in 10 ml THF was stirred overnight at room temperature, and then concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the ethyl acetate solution was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel by eluting with hexane–ethyl acetate (10 : 1) to give 0.45 g (98%) methyl (*S*)-1-(*tert*-butyloxycarbonyl)indoline-2-carboxylate as a colorless oil. This compound was reduced to (**IV**) using lithium aluminum hydride in the same way as described in **1a**. ^{1}H NMR δ: 1.59 (9H, s, CH_3), 2.80 (1H, br, OH), 3.35 (1H, dd, J=16.6 and 9.2 Hz, CH), 3.71–3.76 (2H, m, CH_2), 4.56–4.62 (1H, m, CH_2), 6.95 (1H, t, J=7.3 Hz, phenyl), 7.13–7.16 (1H, m, phenyl), 7.47–7.53 (1H, m, phenyl).

1.4. Ethyl 4-[(S)-(1-n-butylindolin-2-yl)methoxy]benzoate (2a)
To a solution of (**IV**) (0.68 g, 2.7 mmol) in 15 ml CH_2Cl_2 was added triethylamine (0.69 g, 6.8 mmol), *p*-toluenesulfonyl chloride (0.68 g, 3.6 mmol) and 4-dimethylaminopyridine (0.05 g). After stirring for 16 hr at room temperature, the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and the ethyl acetate solution was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel by eluting with hexane–ethyl acetate (5 : 1) to give 1.01 g (92%) of (*S*)-(1-*tert*-butyloxycarbonylindolin-2-yl)methyl *p*-toluenesulfonate.

To a suspension of sodium hydride (0.09 g, 2.2 mmol) in 10 ml DMF at 0°C was added ethyl 4-hydroxybenzoate (0.37 g, 2.2 mmol). After stirring for 20 min at room temperature, the above *p*-toluenesulfonate (0.75 g, 1.9 mmol) was added to the mixture, which was heated for 6 hr at 80°C and then quenched with saturated NH_4Cl solution. The product was extracted with ethyl acetate and the ethyl acetate solution was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel by eluting with hexane–ethyl acetate (10 : 1) to give 0.62 g (84%) ethyl 4-[(*S*)-(1-*tert*-butyloxycarbonylindolin-2-yl)methoxy]benzoate.

A solution of the above compound (0.62 g, 1.6 mmol) in 10 ml CH_2Cl_2 containing 0.7 ml trifluoroacetic acid was stirred for 16 hr at room temperature. The product was extracted with ethyl acetate and the ethyl acetate solution was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography by eluting with hexane–ethyl acetate (2 : 1) to give 0.45 g (97%) ethyl 4-[(*S*)-(indolin-2-yl)methoxy]benzoate as a pale yellow oil.

To a solution of the above compound (0.08 g, 0.2 mmol) in 10 ml DMF was added K_2CO_3 (0.06 g, 0.4 mmol) and *n*-butyl bromide (0.2 g, 1.1 mmol). After stirring for 24 hr at room temperature, the product was extracted with ethyl acetate and the ethyl acetate solution was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel by eluting with hexane–ethyl acetate (15 : 1) to give 0.05g (53%) of **2a** as a pale yellow oil. ^{1}H NMR δ: 0.94 (3H, t, J=7.3 Hz, CH_3), 1.33–1.45 (2H, m, CH_2), 1.38 (3H, t, J=7.3 Hz, CH_3), 1.57–1.69 (2H, m, CH_2), 2.85 (1H, dd, J=16.1 and 7.3 Hz, CH_2), 3.19–3.30 (3H, m, CH_2), 3.31–3.42 (1H, m, CH_2), 4.08–4.15 (2H, m, CH_2), 4.16–4.22 (1H, m, CH), 4.37 (2H, q J=7.3 Hz, CH), 6.41 (1H, d, J=7.3 Hz, phenyl), 6.65 (1H, t, J=7.3 Hz, phenyl), 6.94 (2H, d, J=8.8 Hz, phenyl), 7.00–7.09 (2H, m, phenyl), 8.00 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 73.89; H, 7.60; N, 3.82%. Calcd. for $C_{22}H_{27}NO_3$: C, 74.76; H, 7.70; N, 3.96%.

Compound **2b** was prepared in the same manner as **2a** using benzyl bromide instead of n-butyl bromide.

Ethyl 4-[(S)-(1-benzylindolin-2-yl)methoxy]benzoate (2b)
^{1}H NMR δ: 1.55 (3H, t, J=7.3 Hz, CH_3), 2.82 (1H, dd, J=16.1 and 7.8 Hz, CH_2), 3.24 (2H, dd, J=16.1 and 6.8 Hz, CH_2), 4.03–4.08 (1H, m, CH), 4.09–4.16 (2H, m, CH_2), 4.34 (2H, q, J=7.3 Hz, CH), 4.40–4.54 (2H, m, CH_2), 6.46 (1H, d, J=7.3 Hz, phenyl), 6.64 (1H, t, J=7.3 Hz, phenyl), 6.94 (2H, d, J=8.8 Hz, phenyl), 7.05 (1H, t, J=7.3 Hz, phenyl), 7.08 (1H, d, J=7.3 Hz, phenyl), 7.20–7.34 (5H, m, phenyl), 8.00 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 76.74; H, 6.27; N, 3.69%. Calcd. for $C_{25}H_{25}NO_3$: C, 77.49; H, 6.50; N, 3.61%.

2. Biological evaluation
B. mori (Shunrei×Shougetsu) larvae were reared on an artificial diet as previously reported.[7] The anti-JH activity of compounds was evaluated by the induction of precocious metamorphosis when topically applied to the dorsal abdomen of 24-hr-old 3rd instar larvae as previously described.[3] The JH activity of compounds was evaluated by molting into normal 5th instar when topically applied to allatectomized 4th instar larvae as previously described.[6]

Results and Discussion

Table 1 shows precocious metamorphosis-inducing activity of indole and indoline derivatives against 3rd instar larvae of *B. mori*. 1-Ethylindole analog **1a** had little activity. A marked increase in activity was observed by changing the ethyl to *n*-propyl group (**1b**). The activity of **1b** at 1 μg was low in comparison with that

Table 1. Precocious metamorphosis-inducing activity of KF-13, ethyl 4-[(1-substituted indol-2-yl)methoxy]benzoates and indoline derivatives against 3rd instar larvae of *B. mori*

Compound	Precocious metamorphosis[a] (%)		
	1	10	40 (μg/larva)
KF-13[b]	90	34	12
1a	5	5	5
1b	67	95	90
1c	65	93	94
1d	73	73	84
2a	5	65	82
2b	5	25	28

[a] Values are the average of two experiments. [b] Previously published data.[1]

Table 2. Effects of methoprene, **1c**, **1d**, **2a** and **2b** on the development of allatectomized 4th instar larvae of *B. mori*

Treatment	Dose (μg/larva)	Number of larvae transformed into[a)]		
		Precocious pupa	Larval-pupal intermediate	5th instar larva
Allatectomized control		10	0	0
+methoprene	1	0	0	10
+**1c**	40	0	0	10
+**1d**	40	1	0	9
+**2a**	40	10	0	0
+**2b**	40	5	1	4

[a)] Number of larvae tested: 10.

of KF-13; however, at higher doses, **1b** was more active than KF-13. Butyl (**1c**) and benzyl (**1d**) analogs showed almost the same level of activity as 1b. In contrast to KF-13, the activity of **1b**, **1c** and **1d** did not decrease so much by increasing the applied doses. As previously reported,[3)] the anti-JH activity of KF-13 at low dose levels was entirely due to the (*S*)-enantiomer. We therefore prepared (*S*)-1-butyl (**2a**) and (*S*)-1-benzyl (**2b**) indoline derivatives by starting with (*S*)-indoline-2-carboxylic acid. Both **2a** and **2b** showed lower activity than the corresponding indole derivatives, suggesting that the presence of a basic nitrogen atom is unfavorable for activity.

KF-13 and its analogs have recently been found to exhibit JH activity as well as anti-JH activity;[6)] therefore, we examined whether indole and indoline derivatives had JH activity using allatectomized 4th instar larvae of *B. mori* (Table 2). All allatectomized and acetone-treated control larvae underwent precocious metamorphosis. Methoprene prevented precocious metamorphosis at 1 μg so all treated larvae molted into 5th instar larvae. Indole analogs **1c** and **1d** had obvious JH activity at 40 μg each. It is noteworthy that in contrast to KF-13, **1c** and **1d** having JH activity clearly induced precocious metamorphosis even at high doses, suggesting that the JH activity of **1c** and **1d** was too weak to counteract their anti-JH activity. On the other hand, 1-butylindoline analog **2a** showing precocious metamorphosis-inducing activity at high doses had no JH activity, while 1-benzylindoline analog **2b**, which possessed less precocious metamorphosis-inducing activity than **2a**, showed weak JH activity.

Thus, a new series of 1-substituted indoles (**1b**, **1c** and **1d**), which are conformationally restricted analogs of KF-13, was found to show stronger precocious metamorphosis-inducing activity than KF-13 at high doses. Although these compounds also had JH activity, they might be a structurally novel class of leads for the development of anti-JH agents.

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